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Effect of Adjuvants on the Therapeutic Activity of Dimethomorph in Controlling Vine Downy Mildew. II. Adjuvant Mixtures, Outdoor-Hardened Vines and One-Pack Formulations

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Abstract: Previous investigations have shown that certain C_{12}–C_{18} alcohol ethoxylates with between 5 and 20 moles ethylene oxide gave large enhancements to the therapeutic activity of emulsifiable concentrate (EC) and wettable power (WP) formulations of dimethomorph in controlling two-day-old infections of downy mildew (*Plasmopara viticola*, Berl. & deT) on glasshouse propagated vines (*Vitis vinifera*, L.). In a continuation of that work it has been shown that similar enhancements could also be obtained on vines that had been grown under outdoor conditions for two to three weeks before treatment (outdoor-hardened vines) and it is concluded that the treatments would therefore be effective on field vines.

Mixtures of either ammonium sulfate or the silicone ethoxylate, 'Silwet' L77, with the best alcohol ethoxylates, 'Genapol' C050 or 'Genapol' C080, produced slight antagonism of the action of the 'Genapols' and therefore were of no benefit.

Trials with one-pack formulations prepared containing the 'Genapol' adjuvants produced good dose response curves from which estimated doses for 90% fungal control (ED_{90} values) could be calculated by Logit analysis. These one-pack formulations were significantly more effective than the standard EC formulation (ED_{90} = 335 g AI ha^{-1}, glasshouse vines and 445 g AI ha^{-1}, outdoor hardened vines) with optimum enhancements with those formulations containing an adjuvant: dimethomorph ratio of either 9 : 1 on glasshouse vines (ED_{90} = 13 g AI ha^{-1}) or 6 : 1 on outdoor-hardened vines (ED_{90} = 30 g AI ha^{-1}). This last result was significantly better than that obtained with a commercial formulation of cymoxanil, 'Fytospore', (ED_{90} = 86 g AI ha^{-1}) and encouraged the recommendation that these one-pack formulations should be examined in field trials.

Key words: fungicide, dimethomorph, vine, *Vitis vinifera*, downy mildew, *Plasmopara viticola* adjuvants, formulation

1 INTRODUCTION

Previous trials on glasshouse-propagated vines (*Vitis vinifera*, L., cv. Cabernet Sauvignon) had shown that the therapeutic activity of dimethomorph EC and WP formulations in controlling *Plasmopara viticola*, Berl. & deT could be substantially increased by the use of spray-tank adjuvants.[1] It was shown that C_{12}–C_{14} alcohol ethoxylates with 5–20 moles ethylene oxide were likely to be the most cost-effective and 'Genapol' C050 and C080 were identified as suitable candidates for further investigation. The potential benefits of this improvement could be a widening of the window of application, since early stages of developing infection could also be controlled in addition to infections subsequent to application, a reduction of dimethomorph

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TABLE 1
Composition of Adjuvant-Containing One-Pack Formulations of Dimethomorph

	Ingredient mass, (g)										
	EC Formulation No.										
Ingredients	*I*	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*	*X*	*XI*
Dimethomorph	100	75	75	40	40	25	25	50	50	50	50
Emulsifiers	80	—	60	—	60	—	60	80	80	80	80
'Genapol' C050	—	225	225	360	360	300	300	—	—	—	150
'Genapol' C080	—	—	—	—	—	—	—	150	300	450	—
Solvent	← to 1 litre →										

application rate and an improvement in rainfastness, since foliar-penetrated dimethomorph could not be physically removed by rain. It was therefore important to continue investigation to check whether the adjuvant-enhanced performances were likely to be obtained on field vines and also whether any further improvements were obtainable using mixtures of different types of adjuvant. The experimental procedure used for obtaining the results in the first paper ensured that the improvement in performance was brought about by the adjuvants stimulating penetration of dimethomorph through the adaxial cuticle to the infected cellular tissue beneath the cuticle.[1] It is generally recognised that the leaf cuticles of glasshouse-propagated plant species can be different (usually less substantial) from those on plants grown outdoors. Such differences can result in different performances (usually lower activities) of agrochemicals between glasshouse- and field-grown plants. Since the improvements in dimethomorph therapeutic activity had been brought about by enhanced foliar penetration, it was important to check whether these improvements would be substantially reduced or eliminated by the more substantial cuticles of field-grown vines. This aspect would, of course, be a part of the responses from field trials, but since other factors can also influence field performance it seemed appropriate to obtain early information which focused on this aspect in particular. The information would also be of use in interpreting field trial results. Trials were, therefore, planned in which batches of the small vine plants similar to those used in the previous glasshouse studies would be transferred to a compound outside the glasshouse and allowed to grow on to a similar growth stage to that used previously. These are referred to, in this paper, as 'outdoor-hardened' vines.

There are occasional reports of beneficial effects from

TABLE 2
Effect of Application Rate of Genapol Surfactants on the Therapeutic Control of Two-Day-Old Infections of *Plasmopara viticola* on Outdoor-Hardened Vines by ECI

Adjuvant application rate, (g ha^{-1})	*Fungal control (%)*[a] *Dimethomorph application rate, (g ha^{-1})*					*Mean*[b]
(a) 'Genapol' C050	*6·3*	*12·5*	*25*	*50*	*100*	
0	0	9	0	37	51	19
94	69	63	66	57	66	64
187	57	57	80	74	66	67
375	54	86	80	83	69	74
750	74	77	91	**94**	**94**	86
1500	83	94	94	**97**	94	92
(b) 'Genapol' C080	*6·3*	*12·5*	*25*	*50*	*100*	
0	—	0	0	0	36	9
94	—	28	44	68	76	54
187	—	76	84	92	92	86
375	—	92	92	**100**	92	94
750	—	**100**	92	92	**100**	96

[a] Figures in bold types are those identified as giving >90% control at $P = 0{\cdot}05$.
[b] Mean values of fungal control through the dimethomorph application rate range; these facilitate comparison of the adjuvant treatments.

using combinations of different adjuvants. Indeed, some commercially available adjuvants are supplied in this way. For example, it has recently been shown that mixtures of ammonium sulfate with crop oils or surfactants are better than either component alone when employed as an adjuvant system for sethoxydim,[2] sethoxydim/bentazon mixtures[3] and primisulfuron/bentazon mixtures.[4] Ammonium sulfate can also be an effective sole adjuvant, for example, for glyphosate[5] and imazthapyr.[6] It therefore seemed necessary to check its activity alone and in combination with 'Genapol' C080 on dimethomorph performance. The results from testing 'Silwet' L77, as given in the previous paper,[1] showed that it was a reasonably good adjuvant for dimethomorph, though discounted for further studies by itself on the basis of expense compared with alcohol ethoxylates giving at least equivalent performance. However, silicone ethoxylates are reported as good adjuvants in many situations,[7,8] mainly by virtue of their ability to reduce spray solution surface tensions to values below those of hydrocarbon-based surfactants and to allow the migration of spray solutions to parts of plants (e.g. stomata,[7,9] not reached by other surfactant systems. Although the adaxial surface of vine leaves is astomatous it was felt that an examination of combinations of 'Silwet' L77 and 'Genapol' C080 might be worthwhile, since the low surface tension induced by the organosilicone surfactant might have given some benefit in distributing spray deposits and enhancing dimethomorph uptake and performance, although it was recognised that organosilicone surfactants can also antagonise active ingredient uptake.[10,11] Although adjuvants can simply be added to spray tanks it is more convenient if the appropriate amount of the optimum adjuvant is already incorporated in the commercial formulation to ensure that maximum benefit is always obtained.[12] This paper concludes with the results of testing some early versions of such adjuvant-containing one-pack formulations of dimethomorph.

2 EXPERIMENTAL

2.1 Materials

'Genapol' C050, C080 and 'Silwet' L77 surfactants were obtained from suppliers as noted previously.[1] Ammonium sulfate GPR was obtained from chemical suppliers. Emulsifiable concentrate (EC) and wettable powder (WP) formulations containing no adjuvant were prepared and obtained from Formulation Departments as noted previously;[1] these are referred to in the present paper as ECI and WPI, respectively. Further formulations containing various ratios of adjuvants were prepared by the Formulation Department, Shell Forschung Schwabenheim (courtesy Dr W. Mayer) to the general recipes in Table 1 (ECII to ECXI) and a WP formulation (WPII) to the general recipe, dimethomorph, 100 g; wetting/dispersing agents, 70 g; 'Soprophor' K/202, 600 g; mineral filler, 230 g. 'Soprophor' K/202 (Rhone-Poulenc GmbH, Frankfurt, Germany) is composed of a finely divided silica + alcohol ethoxylate 8EO (2 + 3 by weight).

2.2 Plants and inoculation procedure

Vine plants for the trials carried out under glasshouse conditions, identified in the table headings, were propagated as described previously.[1] The plants used for

TABLE 3(a)
Effect of Ammonium Sulfate and 'Genapol' C080 Combinations on the Therapeutic Control of Two-Day-Old Infections of *Plasmopara viticola* on Outdoor-Hardened Vines by WPI

Adjuvant application rate (g ha^{-1})		*Fungal control (%) Dimethomorph application rate, (g ha^{-1})*					
Ammonium sulfate	*'Genapol' C080*	*6·3*	*12·5*	*25*	*50*	*Mean*[a]	*Overall treatment mean*[a]
0	0	17	35	22	26	28	28
500	0	9	0	4	0	1	
1000	0	0	0	0	42	14	9
1500	0	0	24	0	11	12	
500	94	—	82	87	76	82	
1000	94	—	78	76	71	75	80
1500	94	—	75	89	89	84	
500	187	—	95	89	93	92	
1000	187	—	87	82	84	84	90
1500	187	—	91	93	95	93	
0	375	84	91	89	100	93	93
0	750	95	87	96	98	94	94

[a] Mean values of fungal control through the dimethomorph application rate range; these facilitate comparison of the adjuvant treatments.

TABLE 3(b)
Statistical Significance Levels for the Ammonium Sulfate/Genapol C080 Combinations with WPI

Source	*Pr > F*[a]
Dimethomorph	0·0009***
'Genapol' C080	0·0001***
$(NH_4)_2SO_4$	0·0152**
$(NH_4)_2SO_4$/'Genapol' C080	0·9936

[a] *** Significant at 0·1% level.
**Significant at 1% level (NB negative effect).

those trials identified as outdoor-hardened vines were initially propagated under glasshouse conditions and then transferred to an outdoor compound, under the prevailing May–September Kent weather conditions, at the three- to four-leaf stage. Initially the plants were partially shaded for two to three days to prevent leaf scorch but this shade was then removed and the plants allowed to grow on to the fifth to sixth leaf stage—the same stage as those grown totally under glasshouse conditions. Both glasshouse and outdoor-hardened vines were inoculated, incubated, sprayed and held under glasshouse conditions for four days before being transferred to high-humidity conditions to induce sporulation as described previously.[1]

2.3 Preparation and application of spray solutions

This was carried out as described previously.[1] Where mixtures of adjuvants were used, these were prepared as mixtures with concentrations of both adjuvants at double the concentrations required for application. They were then diluted with an equal volume of dimethomorph formulation dispersion (also at double concentration) immediately prior to application to give the application rates of all components as given in the tables of results. In all cases application was made to four plants, each with two inoculated leaves fully exposed to the spray application.

2.4 Plant treatment, assessment and statistical analysis

Plant treatment and assessment were as described previously.[1] Statistical analysis was conducted using either the *t*-test procedure to detect those treatments giving fungicidal control >90% at $P = 0{\cdot}05$ described previously,[1] or, in the case of the adjuvant combinations, by analysis of variance. However, in the one-pack formulation trials, reasonable dose response trends were observed and these were analysed by a logistic dose–response procedure across dimethomorph application rates for each formulation, assuming parallel responses for the treatments to allow calculation of the estimated dose for 90% control (ED_{90} value) and associated 95% confidence intervals using a SAS program, version 5·08,[13] on an IBM 3431 computer.

3 RESULTS AND DISCUSSION

The effect of 'Genapol' C050 on the performance of the dimethomorph ECI formulation in controlling two-day-old infections of *P. viticola* on outdoor-hardened vines (*V. vinifera* cv. Cabernet Sauvignon) was quite substantial (Table 2(a)). The dose mean values increased systematically with increase of 'Genapol' C050 application rate and >90% control ($P = 0{\cdot}05$) of the disease was

TABLE 4(a)
Effect of 'Silwet' L77 and 'Genapol' C080 Combination on the Therapeutic Control of Two-Day-Old Infections of *Plasmopara viticola* on Glasshouse Vines by ECI

Adjuvant application rate (g ha⁻¹)		*Fungal control (%) Dimethomorph application rate (g ha⁻¹)*				
'Genapol' C080	*'Silwet' L77*	*6·3*	*12·5*	*25*	*50*	*Mean*[a]
0	94	0	31	42	40	28
0	187	63	27	89	77	64
0	375	30	61	56	92	60
94	0	62	0	0	34	24
187	0	47	67	65	78	64
375	0	82	88	71	80	80
94	94	25	36	50	36	37
94	187	60	0	0	57	29
187	94	70	52	87	44	63
187	187	9	60	60	79	52

[a] Mean values of fungal control through the dimethomorph application rate range which facilitate comparison of the adjuvant treatments.

TABLE 4(b)
Statistical Significance Levels for 'Silwet' L77/'Genapol' C080 Combinations with ECI

Source	$Pr > F^a$
Dimethomorph	0·0205*
'Genapol' C080	0·0001***
'Silwet' L77	0·0005***
'Genapol' C080/'Silwet' L77	0·0061**

[a] *** Significant at 0·1% level.
** Significant at 1% level (NB negative effect).
* Significant at 5% level.

obtained with application rates of 50 g AI ha^{-1} dimethomorph and 750 g ha^{-1} 'Genapol' C050. The levels of disease control on these outdoor-hardened vines appeared somewhat lower than those for equivalent treatments on glasshouse-propagated vines.[1] However, since the trials were conducted on separate occasions and variation between trials had been observed, it was more simply concluded, at this stage, that 'Genapol' C050 was an effective adjuvant for dimethomorph on outdoor-hardened and, therefore, field vines.

The conclusion from a trial with 'Genapol' C080 (Table 2(b)), conducted on yet another occasion, was similar and it was concluded that 'Genapol' C080 could also be used for study in field trials. However, before this study was undertaken, combinations of this adjuvant with ammonium sulfate were tested on outdoor-hardened vines, with the trial focusing attention on the effect of ammonium sulfate at zero or low application rates of 'Genapol' C080 on the performance of the WPI formulation of dimethomorph (Table 3(a)). Analysis of variance of the data (Table 3(b)) indicated that fungal control was very significantly related to the presence of dimethomorph and 'Genapol' C080, as clearly seen in Table 3(a), and that there was a significant but negative correlation with ammonium sulfate. That is, with this dimethomorph WPI formulation, ammonium sulfate slightly hindered the low levels of activity at zero 'Genapol' C080 application rate observed in this trial, as made clear by the overall treatment means in the final column of Table 3(a). The systematic increase and actual values of these means with increase of 'Genapol' C080 application rate were similar to those found from the previous trial without ammonium sulfate (cf. Tables 2(b) and 3(a)), despite the fact that the trials had been conducted on different occasions with different types of formulation, giving confidence in the repeatability of the adjuvant enhancement effect of 'Genapol' C080 with all types of dimethomorph formulations.

Such enhancement of the dimethomorph ECI formulation by 'Genapol' C080 was observed in a further trial examining interaction effects of the silicone ethoxylate, 'Silwet' L77, with 'Genapol' C080 (Table 4(a)). Analysis of variance of the data generated in this trial confirmed that increase of application rate of both individual adjuvants brought about enhancements of performance of this EC formulation that were significant at $P = 0·001$ (Table 4(b)). As often observed in this work, increase of fungal control with increase of dimethomorph application rate was less evident but still significant at $P = 0·05$ (Table 4(b)). The analysis confirmed that there was a significant interaction between 'Silwet' L77 and 'Genapol' C080 but, as with ammonium sulfate, the effect of this interaction on enhancement of dimethomorph fungal control was negative, that is, 'Silwet' L77 antagonised the performance of 'Genapol' C080, and the combination was, therefore, of no use. Such antagonism by silicone ethoxylates has been observed with alkylamine ethoxylate enhancement of glyphosate herbicide activity.[10,11] It was therefore clear that the enhancement of dimethomorph performance by alcohol ethoxylates such as 'Genapol' C080 on both glasshouse and outdoor vines was substantial and repeatable and could not be further improved by combinations with other types of adjuvant such as ammonium sulfate or a silicone ethoxylate. A series of one-pack formulations containing either 'Genapol' C050 or C080 was therefore prepared (Table 1) and tested in comparison with the ECI formulation. The extent of fungal control in these trials produced reasonable response curves over the range of dimethomorph application rates and allowed curve fitting by Logit analysis and estimation of application rates for specific levels of control (e.g. ED_{90} : estimated dose for 90% fungal control). As observed in all previous trials, the ECI formulation gave low levels of therapeutic control of the disease with an ED_{90} value of 335 g AI ha^{-1} (Table 5). Incorporation of only 225 g $litre^{-1}$ of 'Genapol' C050 into this formulation, to give ECII with a 'Genapol' C050 : dimethomorph ratio of 3 : 1, improved this therapeutic performance to give a ED_{90} value of 55 g AI ha^{-1} and nearly complete control at 150 g AI ha^{-1} on glasshouse vines (Table 5). Increasing the 'Genapol' C050 : dimethomorph ratio to 9 : 1 (ECIV) gave an even greater enhancement of activity (ED_{90} = 13 g AI ha^{-1}) and complete control at 50 g AI ha^{-1}, but beyond this (ECVI, 'Genapol' C050: dimethomorph = 12 : 1) no further improvement was observed (ED_{90} = 15 g AI ha^{-1}).

Comparison of the results for ECII and ECIII, in which the difference in composition was the incorporation of emulsifiers to aid formulation dispersion on dilution in water, revealed that these emulsifiers could also contribute to the adjuvant enhancement of dimethomorph activity (ECIII ED_{90} = 22 g AI ha^{-1}). At higher 'Genapol' C050 concentrations this additional enhancement was not apparent (cf. ECIV with ECV and ECVI with ECVII) presumably because of the strong effect of 'Genapol' C050.

Of further interest was the result with the WPII formulation containing a partially specified alcohol ethoxylate 8EO in a ratio of 3·6 : 1 with dimethomorph. Its

TABLE 5
Comparison of the Performance of Adjuvant/Dimethomorph One-Pack Formulations with the Standard ECI in Controlling Two-Day-Old Infections of *Plasmopara viticola* on Glasshouse Vines

Formulation[a] *(adjuvant : AI ratio)*	*Fungal control (%) Dimethomorph application rate, g ha^{-1}* 12·5	25	50	100	150	*Calculated ED_{90}*[b] *(g ha^{-1})*	*95% confidence limits, (g ha^{-1})*
ECI	0	0	3	14	62	335a	243–460
ECII (3 : 1)	14	19	89	98	98	55b	48–63
ECIII (3 : 1 + emulsifier)	62	84	90	98	100	22c	19–27
ECIV (9 : 1)	89	97	100	100	100	13d	11–15
ECV (9 : 1 + emulsifier)	78	94	100	100	100	17d	14–21
ECVI (12 : 1)	84	97	100	100	100	15cd	13–17
ECVII (12 : 1 + emulsifier)	92	95	98	100	100	11d	13–17
WPII (3·6 : 1)	21	49	86	95	95	54b	43–68

[a] See Table 1 and Section 2.1.
[b] ED_{90} values with the same letter not significantly different at $P = 0{\cdot}05$.

performance (ED_{90} = 54 g AI ha^{-1}) was significantly better than that of ECI and equivalent to that of ECII, in which the 'Genapol' C050: dimethomorph ratio was 3 : 1, confirming that the adjuvant was equally efficacious with different types of formulation, as observed in the earlier trials.

These results on glasshouse vines thus gave encouragement to test a range of similar EC formulations incorporating either 'Genapol' C080 or 'Genapol' C050 (ECVIII–ECXI), at various adjuvant : dimethomorph ratios, and emulsifiers to give good dispersibility of the formulations in water, on outdoor-hardened vines.

The ECI formulation again gave low levels of activity from which it was calculated that the ED_{90} value was 445 g AI ha^{-1} (Table 6). Incorporation of 'Genapol' C080 at a ratio of 3 : 1 with dimethomorph (ECVIII) gave a similar order of reduction to this ED_{90} value (to 52 g AI ha^{-1}; Table 6) as had been observed on glasshouse vines in the previous trial, indicating clearly that the adjuvant enhancement was obtainable on outdoor-hardened vines with these one-pack formulations. Further increase of 'Genapol' C080 to 6 : 1 and 9 : 1 ratios (ECIX and ECX) gave further enhancements of performance, which was statistically significant for ECIX but not ECX (Table 6).

The result with ECXI, incorporating 'Genapol' C050 at a 3 : 1 ratio with dimethomorph was similar to that incorporating the same ratio of Genapol C080 demonstrating the similar effectiveness of these two adjuvants. The choice between them could therefore depend on other factors such as cost and ease of incorporation into formulations.

All of these one-pack formations gave levels of control in this two-day therapeutic test that were marginally better than that from a commercial formulation of cymoxanil, 'Fytospore' (Table 6). Some of these dif-

TABLE 6
Comparison of the Performance of Adjuvant One-Pack Formulations with the standard ECI in Controlling Two-Day-Old Infections of *Plasmopara viticola* on Outdoor-Hardened Vines

Formulation[a] *(adjuvant: AI ratio)*	*Fungal control (%) Dimethomorph application rate (g ha^{-1})* 12·5	25	50	100	150	*Calculated ED_{90}*[b] *(g ha^{-1})*	*95% confidence limits (g ha^{-1})*
ECI	0	0	0	7	39	445a	333–593
ECVIII (3 : 1)	43	52	80	93	99	52bc	40–68
ECIX (6 : 1)	52	63	87	99	100	30d	23–38
ECX (9 : 1)	55	65	92	98	98	39cd	28–52
ECXI (3 : 1)	26	43	73	99	100	45cd	37–56
'Fytospore'[c]	11	54	85	86	86	86b	60–124

[a] See Table 1 and Section 2.1.
[b] ED_{90} values followed by the same letter not significantly different at $P = 0{\cdot}05$.
[c] Active ingredient application rates are those of cymoxanil.

ferences with ECIX, X, XI were statistically significant in this trial and gave further encouragement that these formulations gave fungal control at application rates comparable to those used for current commercial compounds and therefore warranted examination in field trials.

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